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On the Ultrastructure of Dormant and Active Cambium of Conifers*

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Abstract——The cambium of SUGI (*Cryptomeria japonica* D. DON) and KUROMATSU (*Pinus Thunbergii* L.) with its dormant and active state were compared on the ultrastructural level after glutaraldehyde-osmium tetroxide fixation.

The characteristics observed in the dormant cambium are as follows. 1. The organelles are densely and uniformly distributed throughout cytoplasm. 2. Comparatively many vacuoles are present and they are small. 3. Vesiculated smooth ER are rich, although rough-type ones are sometimes observed. 4. Amoeboid type plastids can scarcely be seen. 5. Reserve substances such as starch granules and lipid droplets are comparatively abundant. In contrast to the dormant cambium the active one is characterized as follows. 1. Cytoplasm is localized at the periphery of cambial cells. 2. Cytoplasm is occupied by a large vacuole. 3. A number of rough ER are present. 4. Amoeboid type plastids are frequently seen. 5. Starch granules and lipid droplets are few.

In addition, cytological differences between SUGI and KUROMATSU are as follows. The cambium of SUGI is characterized by the presence of intralamellar inclusion of some plastids in the dormant state and of phytoferritin of plastids through both states, whereas the cambium of KUROMATSU is characterized by the presence of numerous reserve substances, especially lipid droplets, in the dormant state.

Introduction

It is well known that in temperate region annual rings are generally formed by the difference of seasonal growth of wood. This is ascribed to that the growth of cambial cells stops in winter and are vigorous through spring to summer. Cambium is very important to compare cytological differences between dormant and active states on the ultrastructural level. The comparative analysis of cambium on the ultrastructural level was published by SRIVASTAVA and O'BRIEN (1966),[1] SRIVASTAVA (1966)[2] and ROBARDS and KIDWAI (1969).[3] KIDWAI and ROBARDS (1969)[4] presented more detailed discussions only on the dormant cambium. Ultrastructural observations were recently made on cambial cells under natural and controlled environmental conditions by A. J. MIA (1970)[5] using potted plants. From these investigations, it has been clear that there are characteristic differences in the shape of vacuole and ER, and in the occurrence of reserve substances such as lipid droplets and protein bodies.

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In the present study some of the previous results were confirmed and further characteristic difference in the shape of plastid and the distribution of starch granules in dormant and active cambium were observed. Discussions were made on phytoferritin and cytoplasmic microtubules, and on the cytological differences of these two species, SUGI and KUROMATSU.

Materials and Methods

Materials were obtained from SUGI and KUROMATSU trunks, 40-50 years old, growing in the campus of Wood Research Institute, Uji, Kyoto. Stem blocks including cambium were removed from the trees, wood and bark were cut off and thin zone specimens of cambium including adjacent wood and bark to its either side were cut into proper size for fixation. The small specimens obtained were fixed in 3 or 6 % glutaraldehyde in a 0.05 M phosphate buffer at pH 6.8–7.2 overnight at room temperature or 4°C, and then the specimens were washed with 0.05M phosphate buffer for 4 hrs. The tissue was transferred to 1 % osmium tetroxide in 0.05M phosphate buffer at pH 6.8–7.2. Fixation time was for 4 hrs at room temperature or 4°C. The specimens were dehydrated through a graded aceton series and passed through propylene oxide into a graded propylene oxide/final resin series. Infiltration was carried out overnight and polymerization was done at 40° and 60°C. Sections were cut using glass knives on a Porter-Blum MT-1 ultramicrotome and were mounted on 150 mesh copper grids coated with collodion. All sections were stained on the grids in uranyl acetate and lead citrate (REYNOLDS, 1963).[6] Grids were examined in a JEM T6S electron microscope.

Results

It was previously reported that fusiform cambial cells in SUGI were 3 to 6 cells wide (T. ITOH *et al*, 1968).[7] In the present study they were 4 to 5 cells wide in SUGI and 6 to 8 in KUROMATSU. With the aid of changing pH or adding sucrose, various fixation procedures were tried, and in any cases ray cambial cells were well preserved, while fusiform cambial cells were not well. This was especially the case in active cambium and followings were assumed as a reason; the fusiform cambial cells of conifers are conspicuously long in longitudinal direction; one end is opened by cutting so that cytoplasmic streaming is disturbed.

The organelles, such as nucleus, mitochondrion, plastid, endoplasmic reticulum (including SER and RER), golgi body, microtubule, ribosome, vacuole, and lipid droplet are commonly distributed within the cytoplasm of both species through dormant and active cambium. However, in both states some important differences in shape and occurrence of organelles were observed.

Dormant cambium-Cytoplasmic organelles such as plastid, mitochondrion and lipid droplet were seen within the outer most latewood cells in both species (Fig. 1). The nucleus was located in the center of a cell and several nucleoli were observed within each one. Plastids were found at the periphery of the nucleus and at the same site lipid droplets and mitochondria could also be found (Fig. 2). The shape of nucleus in fusiform cambial cells was ellipsoidal, while the one in ray cambial cells was spherical. Plasmalemma was folded in KUROMATSU. The cytoplasm was occupied with small vacuoles and the other organelles were uniformly distributed in a cell (Fig. 3). Mitochondria had tubular or vesicular cristae and osmiophilic granules (Fig. 4).

Plastids containing starch were rich in ray cambial cells and rare in fusiform cambial cells. Plastid of SUGI contained osmiophilic granules (Figs. 5 and 9), whose amount was greater than that of mitochondrion, net-like structure (Fig. 5), intralamellar inclusion (Fig. 6) and lipid like structure other than starch granules. In addition, particulate aggregations presumed to be phytoferritin granules were found (Fig. 7). The arrangement of this particles were curved in parallel rows. Plastid of KUROMATSU had no intralamellar inclusion and phytoferritin. Vesiculate and cisternal SER were rich in both types of cambial cells (Figs. 8, 9 and 10).

Golgi bodies consisted of 6 to 7 cisternae, around which many vesicles were located (Fig. 13). Lipid droplets encountered frequently were single membrane bound bodies and more or less electron opaque. The organelles of KUROMATSU in ray cambial cells were packed densely (Fig. 11). Ribosomes were seen free in the cytoplasm or as polysomes. Some polysomes were frequently oriented in spiral configulation (Fig. 9). Lipid droplets were observed frequently in the last formed latewood cells and earlywood cells. Microtubules could be seen near the cell wall and not in the cell interior.

Active cambium-Almost all the space of a cell was occupied with a large vacuole and the cytoplasm was pushed aside to the periphery of the wall (Fig. 12). It was conceived that protoplasm streamed actively. The nucleus was suspended with thread of cytoplasm (Fig. 14). There was almost no differences about nucleus and mitochondria between in active and dormant states.

In fusiform cambial cells, plastids containing starch granules could scarcely be seen. In ray cambial cells the amount of these types of plastid were much less than in dormant state and starch granules were small. Immature xylem ray cells had no starch granules in plastids. The plastid of SUGI contained phytoferritin curving in parallel arrays as in the dormant state (Fig. 15). Other inclusions of plastid such as osmiophilic granules and lipid like structure (Fig. 17) were observed. However, intralamellar inclusions were disappeared.

As the morphological feature of plastid the existence of the one called "amoeboid type", which sometimes encircled mitochondrion like structure, was observed (Figs. 14 and 15). ER which was exclusively rough type (Figs. 17, 18 and 19) was observed frequently. Golgi bodies were similar to those in dormant cambium and consisted of 6 to 7 cisternae which had many vesicles (Fig. 12). Lipid droplets were few. In KUROMATSU the organelle was distributed sparsely in the cambial zone cells, whereas a considerable amount of the organelle existed in xylem tracheids.

Two types of microtubules could be seen in the cytoplasm. The one occurred at the periphery of the cell wall as regular arrays, generally but as random arrays in the ray cambial cells (Fig. 17). The other, some of which were bundled, appeared in the interior of the cytoplasm in SUGI (Fig. 18).

Discussion

In both SUGI and KUROMATSU, mitochondria, plastids, lipid droplets are observed in the outer most latewood cells. MURMANIS L. and I. B. SACHS (1969)[8] and P. KIDWAI and A. W. ROBARDS (1969)[4] reported similar results respectively in *Pinus strobus* L. and *Fagus sylvatica* L. The same cytological feature is observed in *Populus* sp. and *Quercus actissima* CARR. (ITOH T.)[9], and it is conceivable that this may be a general feature. This is provably ascribed to that the metabolic activity of a cell decreased in the late growing stage under the influence of external environment.

Comparing fusiform cambial cell with ray cambial one in dormant state, starch granules occur scarcely in the former and a number of starches and lipid droplets can be seen in the latter. There is no visual differences in nucleus and mitochondria between both states.

However, it is primarily noticed that the shape and distribution of vacuoles are different between dormant and active states. In dormant cambium there are a number of small vacuoles, among which other organelles are closely packed. In the active one, however, there is a large vacuole and the cytoplasm is pushed aside to the periphery of a cell.

Secondly the amoeboid type plastid, which is named by NEWCOMB (1967)[10], can seldom be seen in the dormant cambium, while the plastid can be found frequently in the active one. NEWCOMB has found from the examination of serial sections that the cytoplasm is not cmpletely encircled but a part of the plastid is opened and the outer cytoplasm is connected with the inner one. In the present investigation detail observation could not be made and then it is not clear whether the plastid is closed or partly opened. The high metabolic activity is suggested from

the fact that the plastid surrounds partly the cytoplasm. If mitochondrion is actually encircled, it could be recognized as an efficient energy producing system.

Thirdly the difference in the shape of ER must be discussed. It it consistent with previous reports of SRIVASTAVA and O'BRIEN (1966)[1], SRIVASTAVA (1966)[2], KIDWAI and ROBARDS (1969)[3] that vesicular and cisternal SER are rich in dormant cambium, while cisternal RER are numerous in active one. It is reasonable that a number of RER can be seen in the active state. Because the enzymic and structural proteins greatly increase accompaning with cell growth, and ribosomes attached on their surfacies of the ER is engaged in the synthesis and transportation of protein. The occurrence of RER to a lesser extent in the dormant state suggests that protein synthesis and its transportation are more or less active.

It is assumed that a number of SER found in dormant cambium might contain some substances for dormancy or for vernal surge. We must do further cytochemical investigations to clarify the chemical nature of the substance. The occurrence of different form of ER is of great characteristic for distinguishing both states.

Golgi bodies in the dormant state as well as active one have many vesicles at the periphery of the wall.

Microtubules arrange singly or in a bundle of 2 to 3 ones near the cell wall in both states and its orientation is more constant in fusiform cambial cell, while the orientation is random in ray cambial one. The organelles found in dormant cambium might be the remainder engaged in primary wall formation last year. Microtubules can be seen in bundle in the interior cytoplasm in SUGI. In this case a bundle consists of three microtubules. It is very interesting that they are crossed together almost at right angles. It is not distinguished whether these are continued with those near the cell wall.

Intralamellar inclusions of plastids are found in the dormant cambium of SUGI but not in the active one. In SUGI, this is the characteristics of discerning both states. SRIVASTAVA and O'BRIEN (1966)[1] observed the same structural element in *Pinus strobus* L., it could not be found in *Pinus thunbergii* PARL. in the present investigation.

Phytoferritin has been reported only in *Salix fragilis* L. among woody plants (ROBARDS and HUMPHERSON, 1967 and ROBARDS and ROBINSON, 1968)[12]. The ones of that species arrange in fan-shape, which is consistent with present observation in SUGI. However, in *Populus* sp. (ITOH T.)[13] its arrangement is random. According to HYDE, HODGE, KAHN and BIRNSTIEL (1963)[14], the chemical constituents of phytoferritin are similar to the ferritin found frequently in animal cells and are considered as a complex of iron and protein. It is of much interest physiologically

that the complex occurs crowdedly in a local area such as plastids in cambial cells.

As important reserve substances starch, fat and nitrogenous compound have been recognized (ZIEGLAR, 1964)[15]. It is assumed that they occur morphologically as starch granules in plastids, lipid droplets and protein bodies in cambial cells of woody plants. It was also reported that the protein bodies could be seen in cambial cells of dormant state. However, the protein bodies can not be found in the present investigation.

Lipid droplets in KUROMATSU occur not only in cambial cells, but also in the last formed latewood cells, earlywood cells, xylem ray cells and epitherial cells. The fact that KUROMATSU is rich in lipid droplets might suggest that this species belongs to "fat tree" in the cytological point of view.

Abbreviations used are as follows:

FCC=fusiform cambial cell
RCC=ray cambial cell
N=nucleus
M=mitochondrion
P=plastid
GB=golgi body
GV=golgi vesicle
SER=smooth surfaced endoplasmic reticulum
RER=rough surfaced endoplasmic reticulum
V=vacuole
MT=microtubule
LD=lipid droplet
S=starch
PF=phytoferritin
W=cell wall
OG=osmiophilic granule
NLS=net like structure

Fig. 1. Cytoplasmic compornents such as lipid droplet, mitochondrion and plastid remained in outer most latewood tracheid of KUROMATSU. January collection. ×4000.
Fig. 2. RCC of KUROMATSU. The nucleus surrounded with plastids and other cytoplasmic compornents. February collection. ×5000.
Fig. 3. FCC of KUROMATSU. Showing many vacuoles and SER. January collection. ×5000.
Fig. 4. RCC of SUGI. Mitochondria containing vesicular cristae and osmiophilic granules. Showing two types of plastids one containing starch and another phytoferritin. February collection. ×10000.
Fig. 5. FCC of SUGI. Plastids having net-like lamellar structure. January collection. ×19500.
Fig. 6. FCC of SUGI. Plastids having intralamellar structures. February collection. ×16000.
Fig. 7. RCC of SUGI. Showing a number of regularly oriented particles called phytoferritin within a plastid. February collection. ×45500.
Fig. 8. FCC of SUGI. Showing vesiculated SER and a few RER. February collection. ×5000.
Fig. 9. RCC of SUGI. Showing double membrane bound nucleus, SER, RER, lipid droplets,

golgi bodies and plastids. The last has several osmiophilic granules and starches (×6600). Inset shows spiral arranged polysomes (×20500). February collection.

Fig. 10. RCC of SUGI. Showing cytoplasm occupied densely with vesiculated SER. January collection. ×10000.

Fig. 11. RCC of KUROMATSU. Reserve substances such as lipid droplets and starches are densely packed. February collection. ×14800.

Fig. 12. RCC of SUGI. Cytoplasm is located at the periphery of a cell. Showing golgi bodies having 6 cisternae. May collection. ×21000.

Fig. 13. FCC of SUGI. Golgi bodies consist of 6 cisternae bearing many vesicles. February collection. ×49000.

Fig. 14. RCC of SUGI. Nucleus surrounded with the thread of cytoplasm. May collection. ×5000.

Fig. 15. FCC of SUGI. Showing amoeboid type plastid, which bear phytoferritin, including cytoplasm. April collection. ×24500.

Fig. 16. RCC of SUGI. Mitochondria surrounded with plastid. May collection. ×15800.

Fig. 17. RCC of SUGI. Showing numerous rough type ER and plastid containing lipid like structure. May collection. ×15800.

Fig. 18. RCC of SUGI. Showing microtubules near the cell wall. May collection. ×15800.

Fig. 19. RCC of SUGI. Showing cytoplasmic microtubules on the upper right and lower left. These are oriented parallel to the plane and are a bundle of three ones (×17000). Inset showing three microtubules bundled. The bundle is parpendicular to the plane (×66000). May collection.

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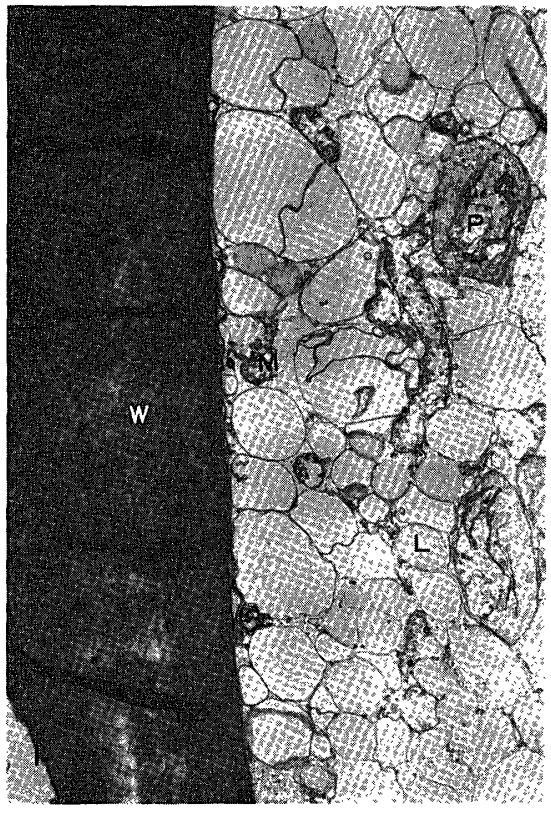
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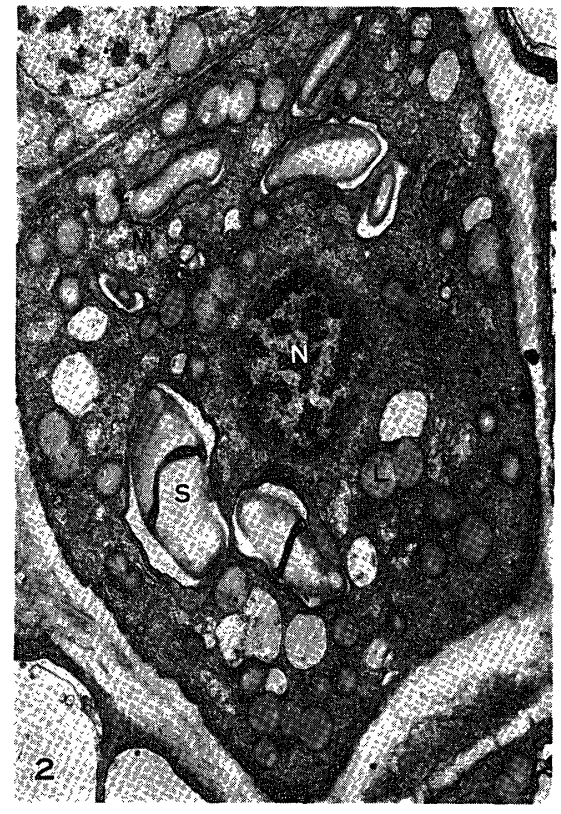
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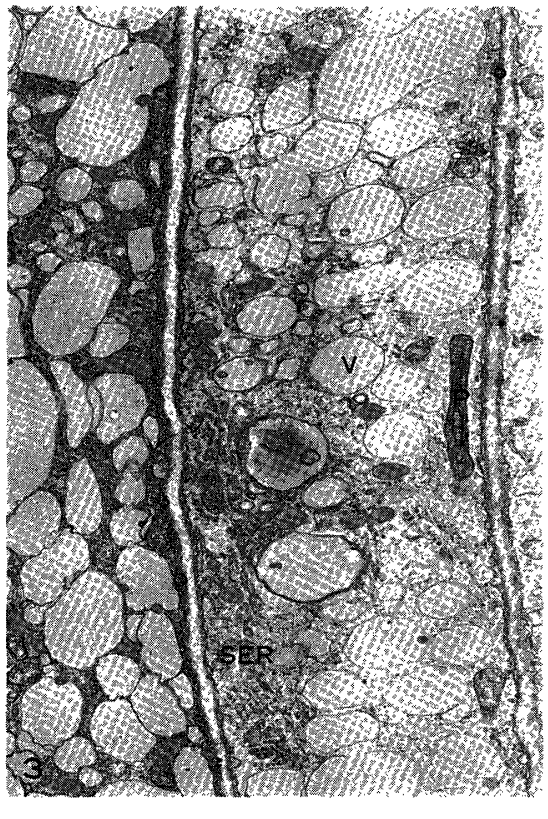
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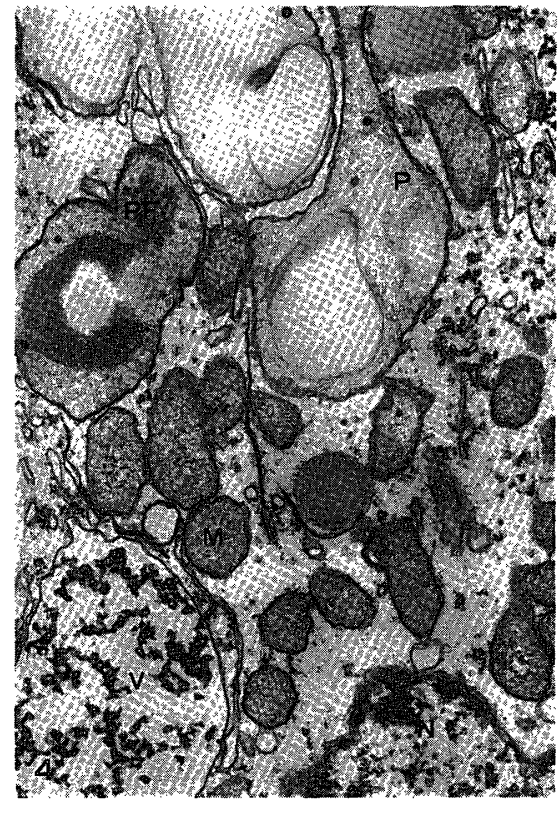
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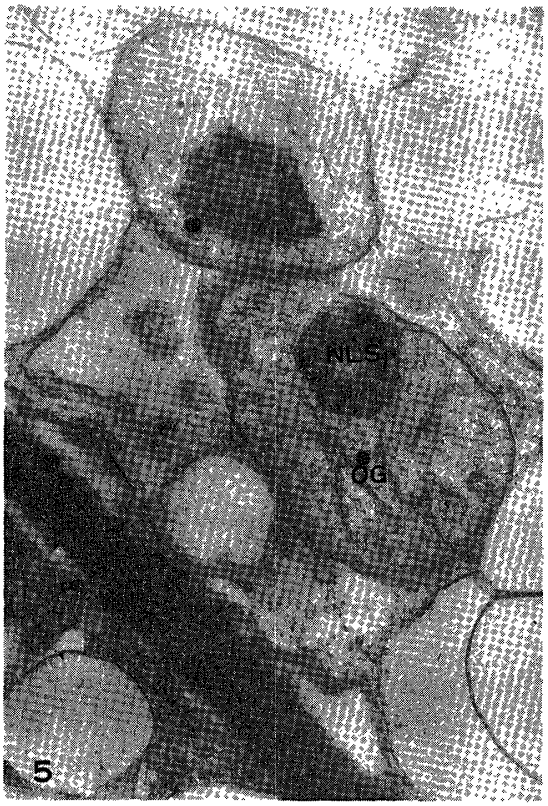
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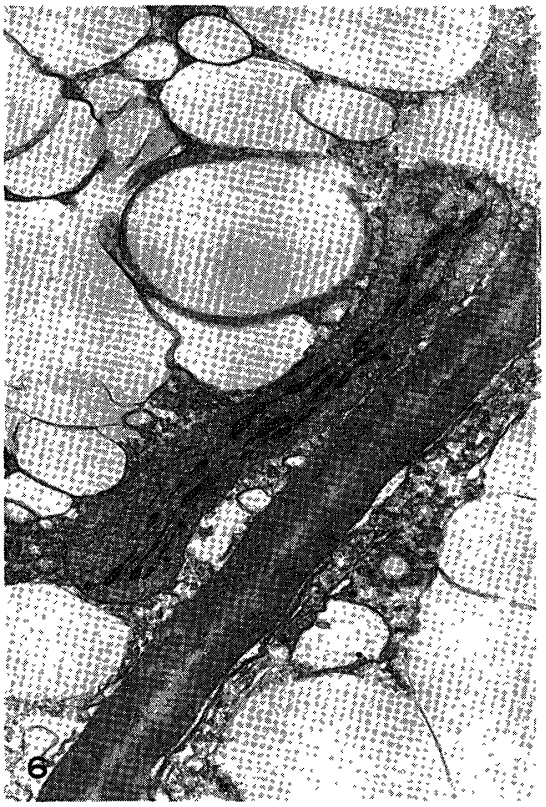
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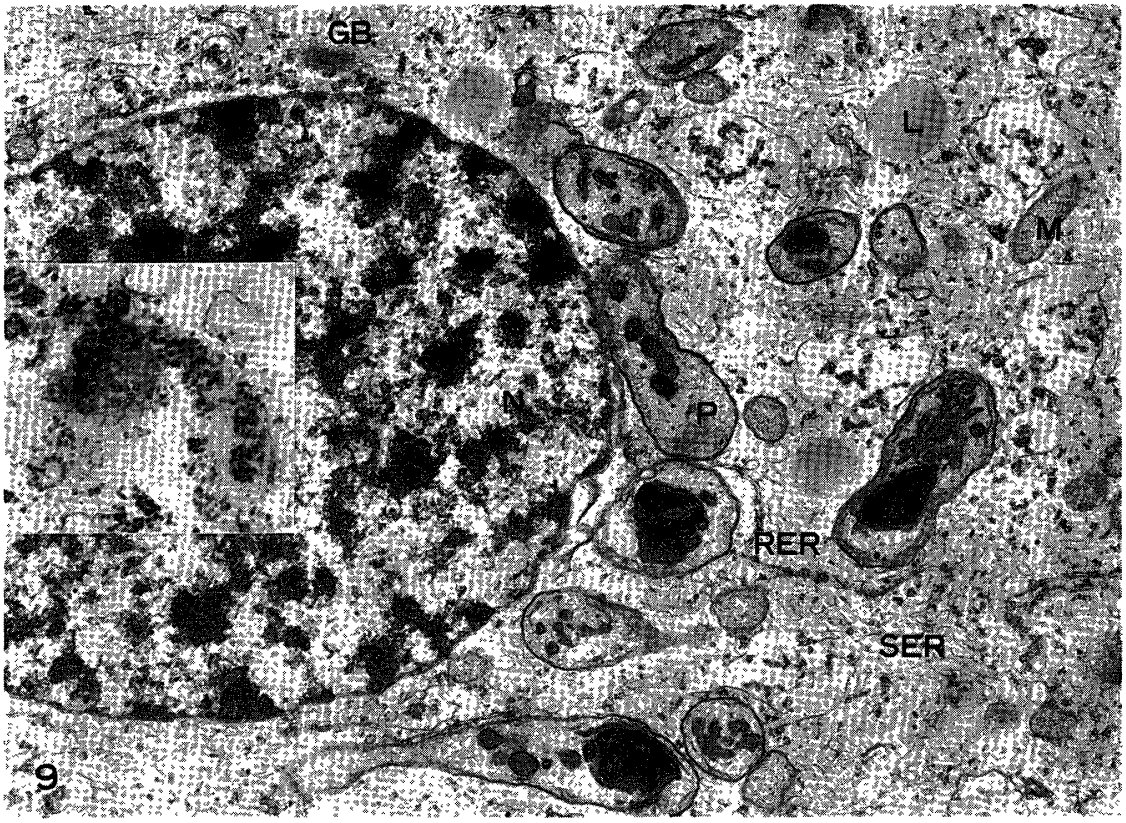

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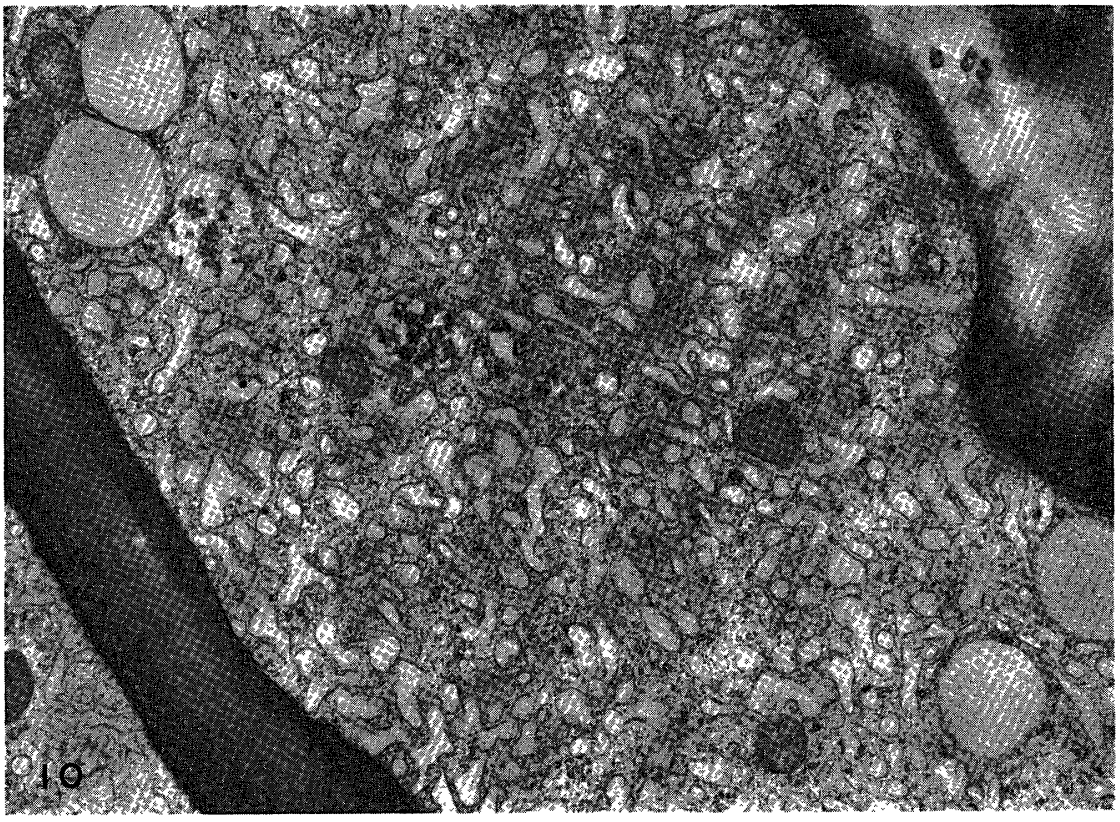

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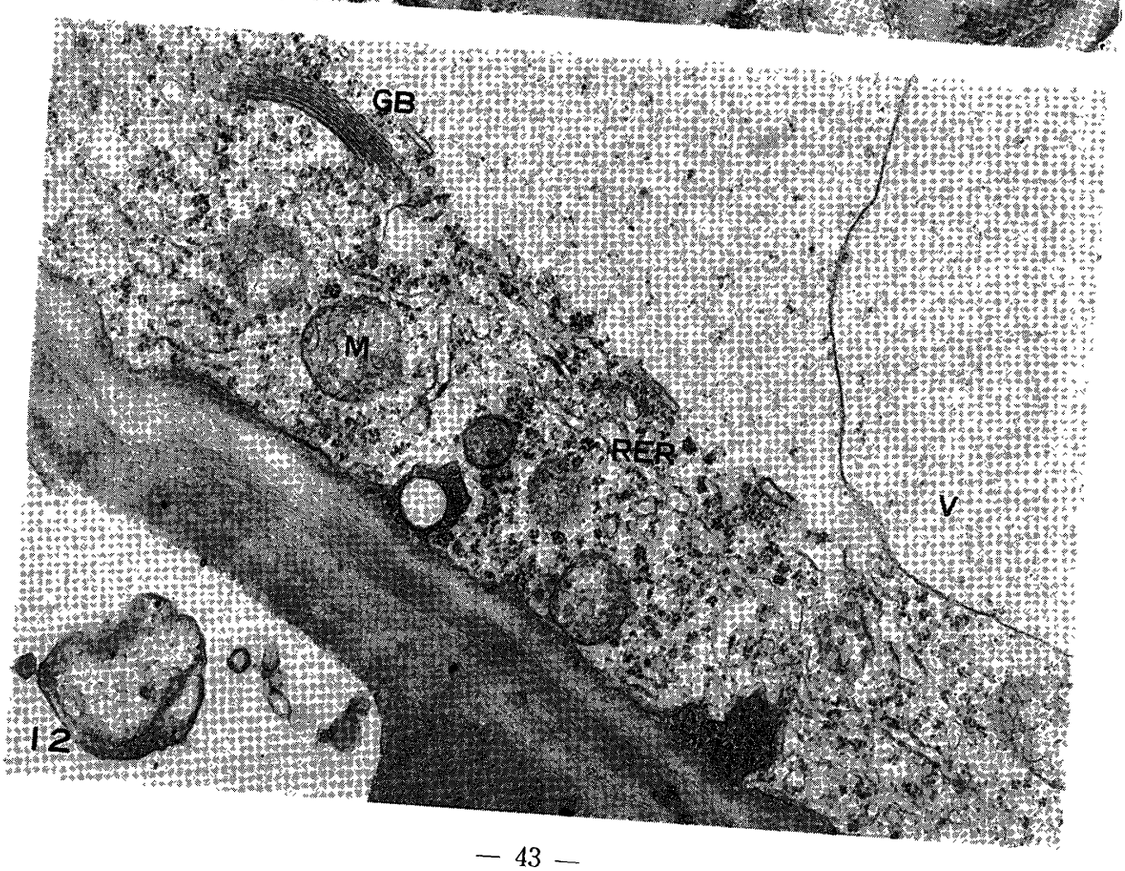
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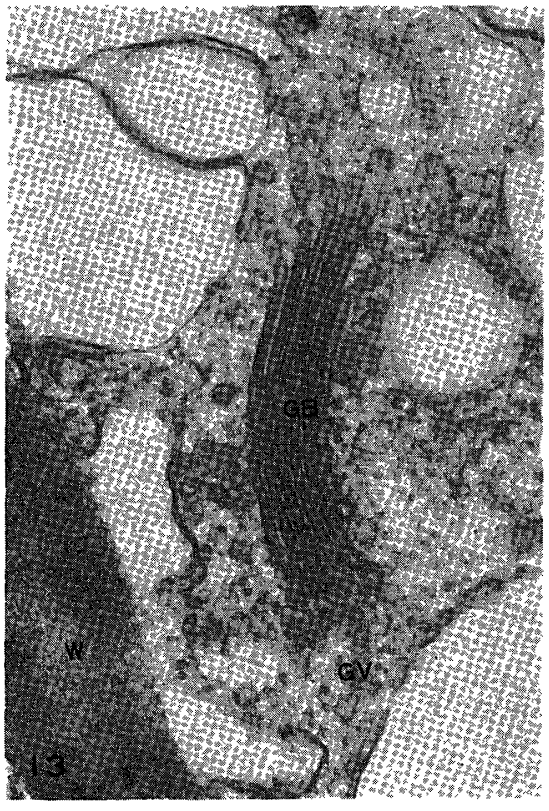
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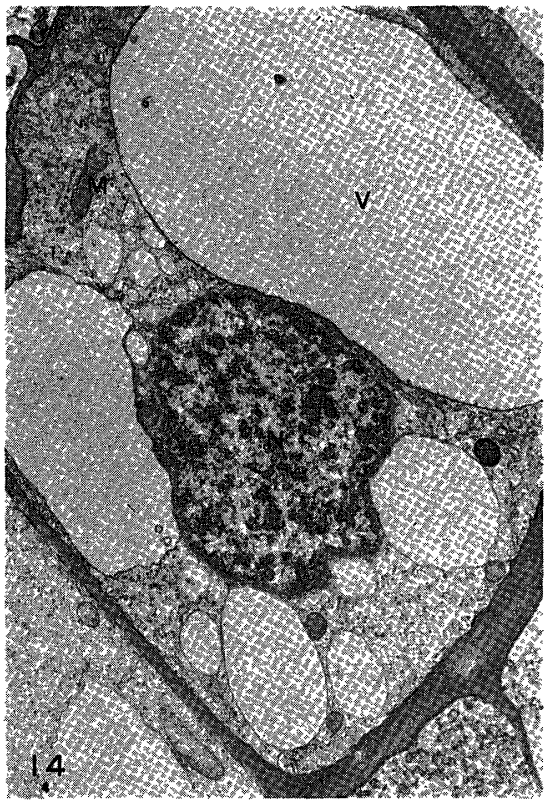
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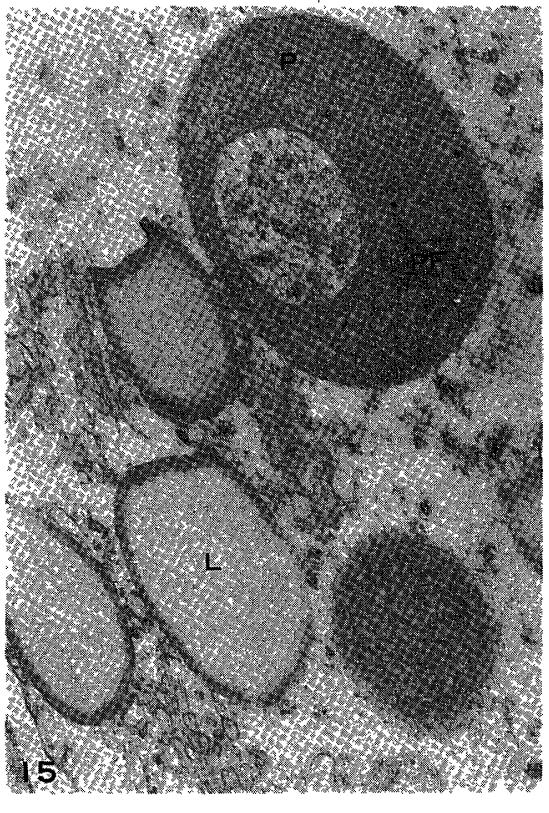
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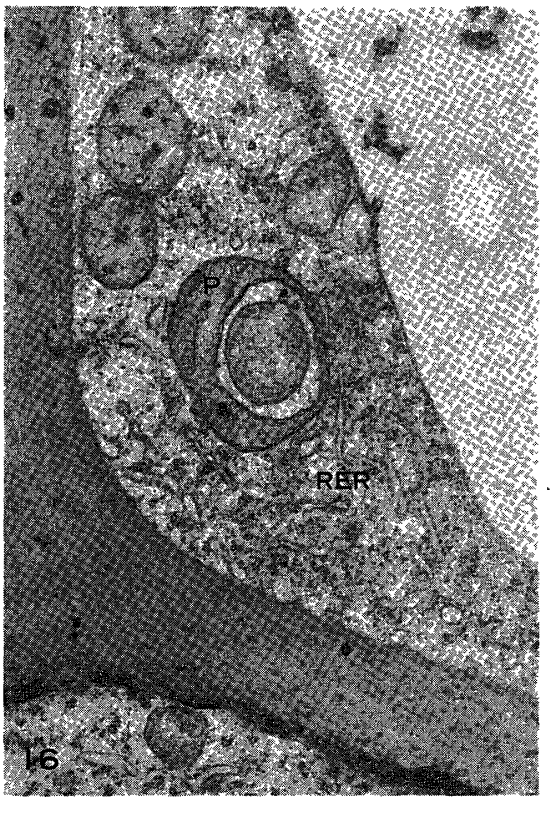
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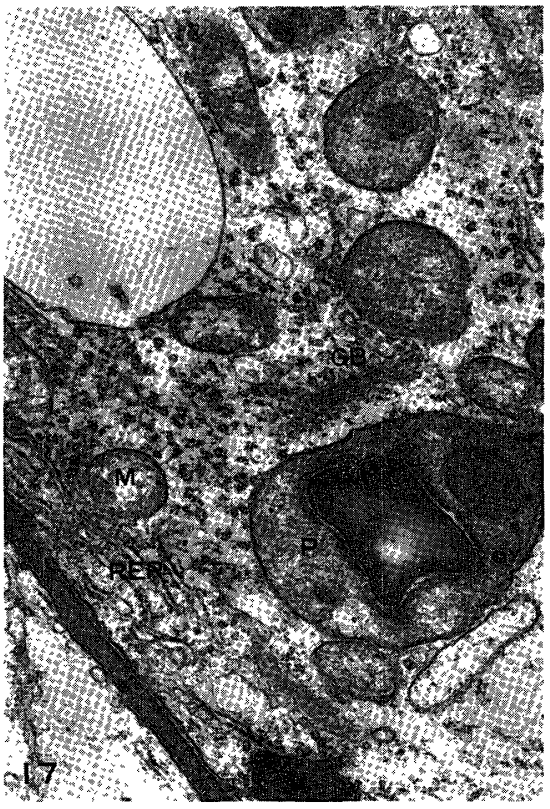

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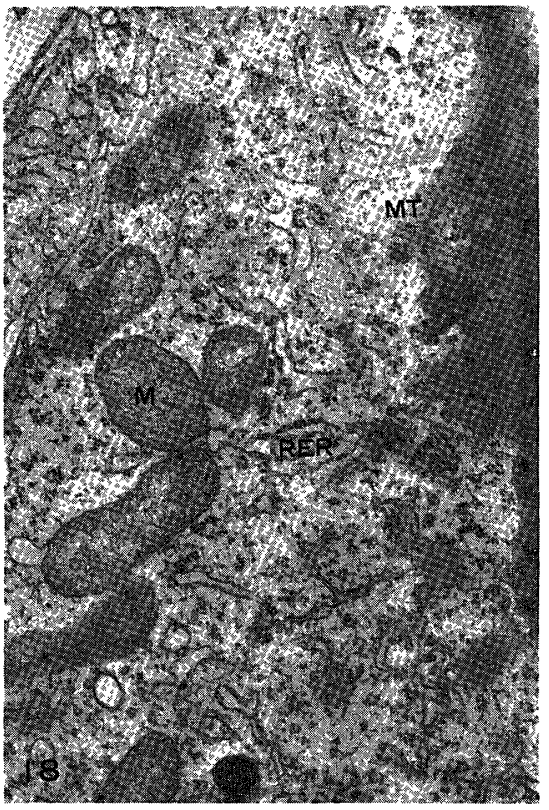

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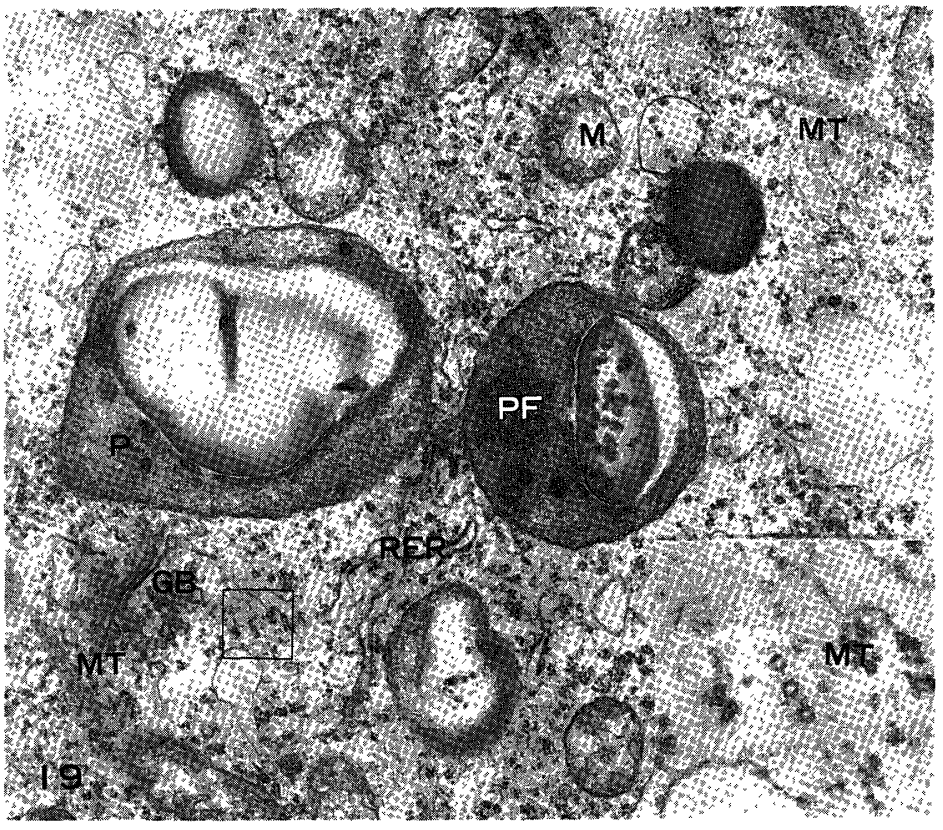

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